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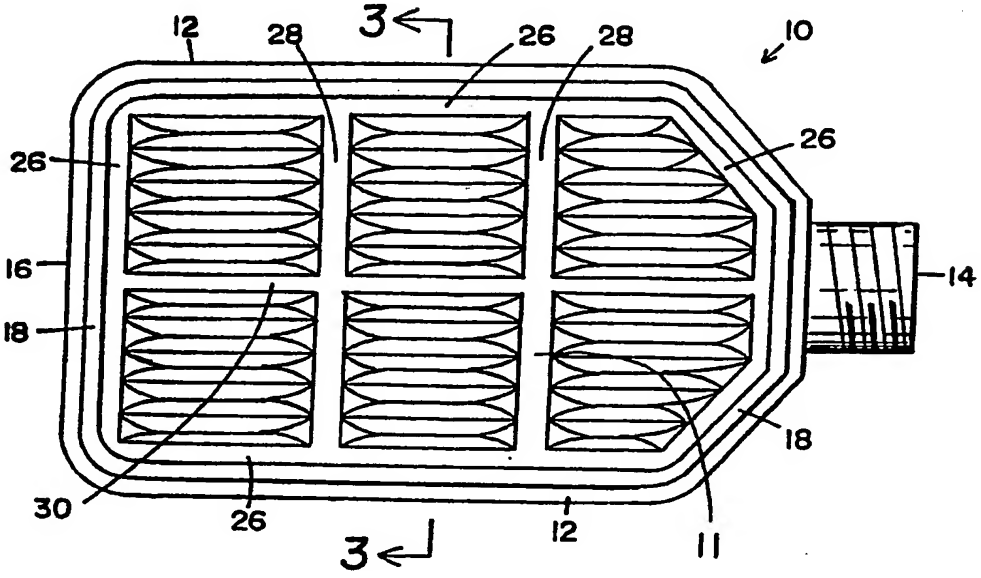
(54) Title: CELL CULTURE FLASK



(57) Abstract

A laboratory flask (10) for confluent growth of animal cell cultures in which the bottom surface (11) contains troughs or channels such as formed by folds (32) to provide increased growing surface. Flat regions (26, 28, 30) allow visual inspection of the cells and also structuraliy reinforce the bottom wall. A flat area (34) may be disposed between each corrugation or pleat (32). Corrugations (32) can be surrounded by pannels (26, 28, 30) to form regions. Cells can be harvested by cutting rims (18, 20) which are thinner than the walls of the flask to gain access to the cells adhered to the pleated bottom surface of the flask.

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CELL CULTURE FLASK

Technical Field

This invention in the biotechnology field concerns improvements in the flasks within which cell cultures are cultivated. More specifically, a flask is described that has an enhanced surface area upon which to grow cells without compromising necessary structural characteristics. Also, the flask is blow molded in such a way as to create a hollow corner bead that is both thinner and stiffer so as to provide an easy place in which to cut the flask open for full access to the interior thereof.

Background of the Invention

It is useful and desirable to grow as many cells as possible in a controlled environment for research purposes or to obtain the cell by-products therefrom. This is routinely done in laboratory flasks into which some cells are introduced. The cells attach to and grow on the bottom wall of the flask, immersed in a suitable sustaining media. The flask is kept in an incubator to maintain it at the proper temperature.

Typically, many flasks are stacked together in the incubator and a number of cultures are simultaneously grown. Small variations in the growth medium, temperature, and cell viability have a pronounced effect on the progress of the cultures. Consequently, repeated microscopic visual inspections are needed to monitor the growth of the cells. These inspections involve carefully removing a flask from the incubator, keeping it level, and placing it on an inverted microscope. The microscope can be focused on the cell layer inside the transparent bottom wall of the flask so as to permit a detailed examination.

Attachment cells, the target culture of the present invention, need a suitable surface which they can attach to before they can multiply and creat a layer over the full surface area available. When the surface is fully occupied

with a monolayer of cells, further growth is blocked. Thus, the production of cells or cell by-products is limited. Using more flasks has the disadvantage of requiring more handling, more microscope examinations, more risk to the flask contents in the form of contamination or physical damage, and more expense. If each flask could have a greater growing surface area, productivity would clearly be improved. But any change of the flat horizontal growing surface has been regarded as unworkable for a number of reasons.

Firstly, if the growing surface is not flat, it is not possible to focus the inverted microscope on the very thin monolayer of cells thereon. Secondly, if the surface is not flat, particularly sensitive cells may not grow uphill on the non-horizontal surfaces. Furthermore, the repeated thermal changes resulting from moving the flasks in and out of the incubator for inspections tend to warp and distort the growing surface which disturbs the cells and complicates the task of focusing the microscope. This problem is exacerbated if the surface is not flat. Still another problem is encountered when the time comes to remove the contents of the flask. Non-flat growing surfaces trap the contents in the convolutions, making access and removal laborious and difficult. The present invention strives to overcome all of the above disadvantages.

Statement of the Prior Art

A typical example of the prior art is shown in United States Patent 4,770,854 to Lyman, which discloses a rectangular culture flask having a neck shaped and angled to optimize access to the interior. The bottom wall 16, where growth normally takes place, is flat in accordance with the prevailing wisdom of the prior art. A variation on the above theme may be seen in United States Patent Des. 285,725 to Franchere, which has the neck located in the corner, but still retains the flat bottom needed for optical examination.

United States Patent 4,824,787 to Serkes et al. shows not a flask, but a cell culture container in a related art. Serkes et al. describes a roller bottle for the growing of cells in more of a high production environment. Cells that have been developed or selected to be more hardy are introduced into a roller bottle that revolves continuously during incubation, keeping the full interior wall of the bottle periodically bathed with nutrient solution. These hardier cells can withstand turbulence, movement, and general disruption. Thus, the walls can be corrugated to increase the growing area and yield. The cells can still attach to these corrugated walls since the cells are generally more rugged. The transfer of these corrugations, however, to the research type rectangular flask has been thought to be a poor idea, since the cells used in research need a horizontal surface upon which to grow well. Corrugations make practically all of the growing surface nonhorizontal all of the time, impeding attachment and growth. This disadvantage is made worse by the more finicky nature of research cells which include, for example, primary cells taken directly from animals for study and neuron type cells that are particularly hard to induce to grow. This invention discloses a flask design with increased growing surface for the more fragile cells as well.

Summary of the Invention

Briefly, the flask of the present invention incorporates a growing surface that blends a balanced combination of corrugated areas, to increase the surface area upon which cells may grow, and flat areas intermixed with the corrugated areas to permit microscopic inspection of the cell layer. The flat areas are dispersed in such a way as to insure that any given growing area is proximate to an inspection area so that a comprehensive and representative examination is possible, despite the fact that the majority of the flask growing surface is not accessible to the microscope. In addition, the flat areas serve to maintain structural integrity across the whole of the growing

surface. Without the flat areas, the corrugations introduced into the flat flask wall would weaken it excessively, allowing the flask to expand when warmed in the incubator. Such expansion makes stacking in the incubator impossible, and use of the microscope impossible. Another improvement stems from orienting the corrugations with their fold lines pointing toward the flask opening, to make removal of the contents through the opening easier and more complete.

Since removing the contents through the regular flask opening can be quite tedious and time consuming, the flask of this invention is specially molded, using blow molding techniques, to form a thin, hollow, semicircular rigidized retaining rim bead about the peripheral edge of both the flat and corrugated faces of the flask. This rim affords a somewhat self-guiding cutting path along which a sharp knife can be drawn to cut the entire face out of the flask for easier access to the interior of the flask. The thinner material in the bead allows easier penetration by the knife than in the surrounding sides and face of the flask, so that the knife tends to follow the thinner path of least resistance. The semicircular shape of the peripheral bead rigidizes it so that it can resist the pressure of the knife and cut cleanly, rather than distort away from the knife blade. The semicircular shape also creates side walls that are parallel to the knife blade, which also assist in guiding the blade along a straight path at the very edge of the flask face. Further details of this invention, along with additional benefits and advantages, are explained hereinafter and with reference to the drawings.

Brief Description of the Drawings

Figure 1 is an elevational view of the flask of the present invention as seen from the bottom or growing surface side, showing the fluted or corrugated areas and the intermixed flat areas. Also shown is the peripheral thin semicircular bead that allows easy cutting of the fluted side of the flask.

Figure 2 is a view of the generally flat opposite side of the flask from that shown in Figure 1 showing the top surface and the stacking risers thereon and the thin semicircular retaining rim that permits easy cutting of the flat side of the flask.

Figure 3 is a sectional view taken on line 3-3 in Figure 1 that further illustrates the important features of the flask.

Figure 4 is a sectional fragmentary view, similar to the bottom part of Figure 3, showing another shape for the corrugated growing surface with flat areas between each pair of flutes.

Figure 5 is a fragmentary elevational view, similar to Figure 1, showing the embodiment of Figure 4 with additional flat areas between the flutes.

Figure 6 is an enlarged fragmentary perspective view of a typical thinned semicircular section of the flask, created with blow molding techniques, showing how the face of the flask may be cleanly and evenly cut away to provide maximum access to the inside of the flask.

Detailed Description of the Invention

In Figure 1, the flask 10 of the present invention is shown from the bottom side 11 which is the side upon which cells are normally cultured. The flask includes edge walls 12, a threaded opening 14 adapted to receive a conventional screw cap, and an end wall 16 opposite opening 14. Flask 10 is more or less rectangular to facilitate efficient and compact stacking in the incubator. To avoid any chance of leaks, flask 10 is preferably blow molded from a single piece of transparent plastic. Any plastic from the polyester family may be used to blow mold a flask having the desired structural characteristics optimized by the configuration of the present inventive flask. However, a particularly good choice for this plastic is polyethylene terephthalate with a glycol additive (PETG), an amorphous therm plastic that not only has good forming properties but also has been found to have acceptable cell attachment and

release characteristics. Also, this plastic can withstand sterilization by gamma radiation without physical and chemical degradation. This plastic is commercially available from Eastman Chemical under the trademark Kodar 6763. PETG, being a softer polyester resin, is more easily cut with a knife than typical prior art plastics, such as polystyrene. This attribute is useful provided the flask is shaped as shown in Figure 3, with semicircular rims 18 and 20, that provide a rigid area of the flask that will not distort under the pressure of a knife.

The bottom fluted growth face 11 of the flask is surrounded by a peripheral raised rim or bead 18 that is also visible in the cross section of Figure 3. The non-fluted face 13 of the flask, opposite from the fluted face 11, and shown in Figure 2, also has a peripheral rim 20 that is slightly larger than rim 18. Edge walls 12 slant out as shown by portions 22 in Figure 3, so that the flasks can be nested or stacked with rim 18 coming to rest just inside rim 20 and on a group of four platforms or risers 24. Risers 24 maintain a small clearance between stacked flasks, so that if flat face 13 is bowed upward by internal pressures generated during incubation, the stack is not upset.

The shape of rims 18 and 20 is shown in greater detail in Figure 6. Flask 10 is formed by blowing air into a tube of PETG plastic so as to expand it out to a surrounding shape-defining mold that forms the exterior surface of the flask. Rims 18 and 20 are defined by recesses in this mold surface. As the bubble of plastic reaches these recesses, the layer of plastic bridging the recess stretches up into the recess, becoming thinner in the process. Figure 6 shows that the face 40 of the flask and the side wall 42 of the flask are both thicker than the semicircular raised rim or bead 44. At the same time, the curved shape of bead 44, and the vertical walls 46, combine to rigidize bead 44 so as to hold it in place against the pressure of a knife 48 drawn therealong. Bead 44 is thus both thinner and stronger at

the same time, making it easier to cut along a straight line just at the edge of the faces 11 and 13. A straight cut path 50 is assured by several factors. Firstly, the knife tends to follow the route of least resistance through the thinner plastic in the semicircular bead. Secondly, the flat side walls 46 are parallel to the flat side of blade 48 (blade 48 is much larger in reality than it is drawn in Figure 6), and thus help guide blade 48 along the edge for a clean straight cut. Finally, the semicircular shape of bead 44 allows the cut material to spread easily out of the way of the blade, as shown by spreading cut 50, so that the blade 48 does not bind in the narrow cut.

Alternatively, one may draw the blade 48 along the inside edge 52 of the bead 44. But this provides a slightly smaller opening, the plastic may be thicker and harder to cut, and the rigidizing strength of side wall 42 is less helpful.

Conventional flasks, with flat faces on both sides, can make use of the easily cut rim 44 to allow full and complete access to the interior of the flask. But enhanced growing area flasks, with corrugations on the bottom, of the type described hereinafter, benefit even more from easy to cut away faces. Corrugated flasks have recesses between the flutes where cells are hard to retrieve working through narrow opening 14. If desired, the fluted side may also be cut away, to facilitate studying the cells in place, or permanently storing the cells, as grown, for future reference.

Referring to Figures 1 and 3, it may be seen that the bottom wall of flask 10 has a mixture of flat and corrugated areas. Six approximately square areas of corrugations are defined by flat areas around and between the square areas. Since the flask shown is about 3 by 4 inches, the square areas are about an inch square. Larger flasks would have more square areas so as to keep the square areas about the same size. The corrugations increase the growing surface area, while the flat areas provide an optically flat trans-

parent wall where a microscope can be positioned and focused to examine the cells inside the flask. The flat areas have other functions as well. A peripheral border flat zone 26 is formed just inside rim 18. This border flat zone laterally stiffens the surface at the edges and keeps the rim 18 straight to insure proper stacking of the flasks, and also keeps it straight during cutting of rim 18. Thermal stresses might otherwise distort the flask rim as it is moved in and out of the incubator.

A pair of flat cross band areas 28 are formed that extend from the border flat on one side to the border flat on the opposite side. Cross bands 28 comprise tension members that prevent the flask from expanding outward in response to internal pressure in the flask. In use, the flask is sealed and warmed in the incubator. Warming raises the pressure inside the flask, typically about 1 psi. The flask walls are very thin and the folds weaken it considerably in the direction perpendicular to the folds. Hence, internal pressure in the flask easily bows the bottom and edge walls outward. This bowing is resisted by tension bands 28. Without restraint, the bowing could prevent stacking and distort the flat areas enough to make examination by microscope impossible.

A third flat area 30 is formed from the border flat near opening 14, generally down the center of the flask bottom, to the border area near end 16. The combination of flat areas 26, 28, and 30 divide the flask bottom into six corrugated areas, each filled with a series of folds 32 that significantly increase the surface area for cell attachment and growth. The surface area is about doubled with the fold shape shown in Figure 3. Deeper folds would, of course, give even more area, but tend to trap the contents in the deeper crevices. On larger flasks, additional flat areas would be needed to define more than six corrugated areas, keeping the corrugated area the proper size.

One of the strongest contributors to variations in cell growth are temperature gradients in the incubators. Most

often, one sees a different progress at one end of the flask relative to the other end. Reducing the distance from one flat viewing area to the next lessens the probability of overlooking a variation in the effect of the temperature. The arrangement of flat viewing areas 26, 28, and 30 affords a balanced mixture of flat and corrugated areas that optimizes growing surface but still insures that any location on the bottom wall is only a short distance from a flat viewing area. Accordingly, the cells that may be microscopically examined are likely to comprise a representative sample of the condition of all cells in the flask.

When cell cultures are more uneven in their growth rates, it may be desirable to provide even more viewing areas. In Figures 4 and 5, another embodiment of the invention is illustrated wherein flat viewing areas are introduced between each pair of individual corrugations. Figure 4 is a section of the growth surface that may be used instead of the folds 32 shown in Figure 3. Each pair of folds 32A has a flat viewing area 34 formed therebetween to provide an inspection zone proximate every fold. A portion of the resulting bottom growth surface is shown in Figure 5. This embodiment of the invention provides a great increase in inspection area but, of course, some reduction in growing surface.

The folds in each corrugated area are oriented parallel to edge walls 12 so as to create grooves or channels that point toward and convey fluid to the opening 14 to make removal of cells or cell by-products through opening 14 easier. Channels oriented in a different direction would trap the valuable contents of the flask, making removal very difficult. However, this necessary orientation causes the bottom wall to be susceptible to the bowing effect discussed previously. Hence, the tension strap function of flat areas 28 provides a synergistic benefit, in addition to the primary function of providing a viewing location. For even

easier removal, however, the rim may be cut as described in Figure 6 to totally expose the interior of the flask.

Widths, lengths, and spacings of the flat viewing areas are controlled, in part, by the flat area requirements of the microscope, the overall flask size, and the cell growth characteristics. Therefore, the particular arrangement of flat areas shown in the drawings is not critical. Numerous variations may be seen to fall within the scope and spirit of the invention. Limitation of the invention is solely determined by the appended claims and their equivalents.

CLAIMS

1. In a stackable, generally rectangular, cell culture flask having a top wall and a bottom wall connected to side walls and end walls, one of said end walls including a neck and closure, the improvement comprising:

an extended surface area bottom wall having an interior surface capable of attachment and confluent growth of animal cells, said bottom wall containing a plurality of parallel, non-horizontal folds and at least one flat viewing panel disposed adjacent to said folds and said folds comprising a majority of the interior surface of said bottom wall whereby the surface area for cell growth of said bottom wall is significantly increased.

2. A cell culture flask according to claim 1 in which the folds have a longitudinal axis, the bottom wall contains a plurality of flat viewing panels and a portion of the flat viewing panels are disposed parallel to said longitudinal axis of the folds.

3. A cell culture flask according to claim 2 in which a portion of the flat viewing panels are disposed transverse to the longitudinal axis of said folds.

4. A cell culture flask according to claim 3 in which the flask has an axis parallel to said side walls and the longitudinal axis of said folds is disposed parallel to the axis of the flask.

5. A cell culture flask, according to claim 3, in which a plurality of parallel folds are disposed adjacent to each other to form at least one region.

6. A cell culture flask, according to claim 2, in which a flat panel is disposed adjacent each fold.

7. A cell culture flask, according to claim 2, in which each fold has a lowermost edge and substantially all of said edges are disposed in a common plane.

8. A cell culture flask, according to claim 2, further comprising:

a region proximate to the periphery of at least

one of said walls which region is shaped to form an easier to cut path along which a sharp tool can be drawn to cut and detach the entire wall of the flask to permit improved access to the interior of the flask.

9. A flask according to claim 8 in which said region comprises a thinner material so as to be easier to cut.

10. A flask according to claim 8 in which said region is rigidized in a direction generally perpendicular to said one of said walls so as to better maintain its shape under the applied force of said sharp tool.

11. A flask according to claim 8 in which said region is curved away from and back toward said one of said walls.

12. A flask according to claim 8 in which said region comprises a generally semicircular shaped, thinner, portion of the intersection of said one of said walls with the other walls connected to said one of said walls.

13. A flask according to claim 8 in which said region is formed along the periphery of both said top and bottom walls to permit easy cutting and removal of the top and bottom walls of the flask.

14. A flask according to claim 8 in which said region is formed by blow molding said flask inside a surrounding mold with recesses in the mold at the locations corresponding to said regions so that the flask walls are stretched into the recesses creating said region with thinner material than the rest of the flask.

15. A flask of claim 14 in which said flask is blow molded in one piece to reduce the possibility of leaks and contamination of the contents of the flask.

16. A method of growing cells in a cell culture flask according to claim 8, comprising the steps of forming said flask with easy to cut regions in the surface of the flask, introducing animal cells into the flask, maintaining nutrient and temperature conditions to encourage confluent growth on the interior surface of the flask, and removing a part of the wall of the flask by cutting along the easy to cut regions so as to access the contents of the flask.

17. A method according to claim 16 in which the step of forming the flask comprises blow molding the flask into a mold with recesses in the mold surface so as stretch the flask wall, in the easy to cut regions, into a thinner, curved, and more rigid shape.

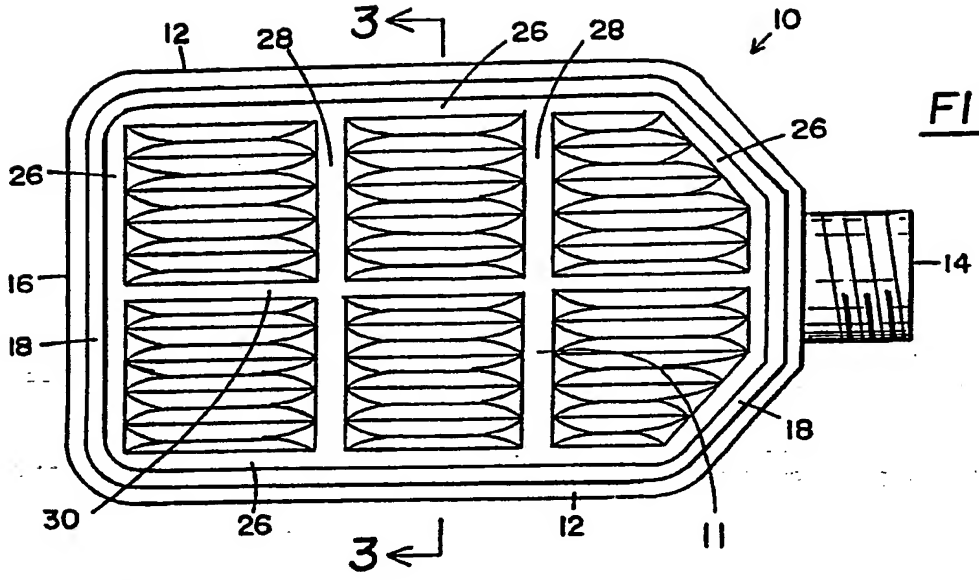
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FIG. 1

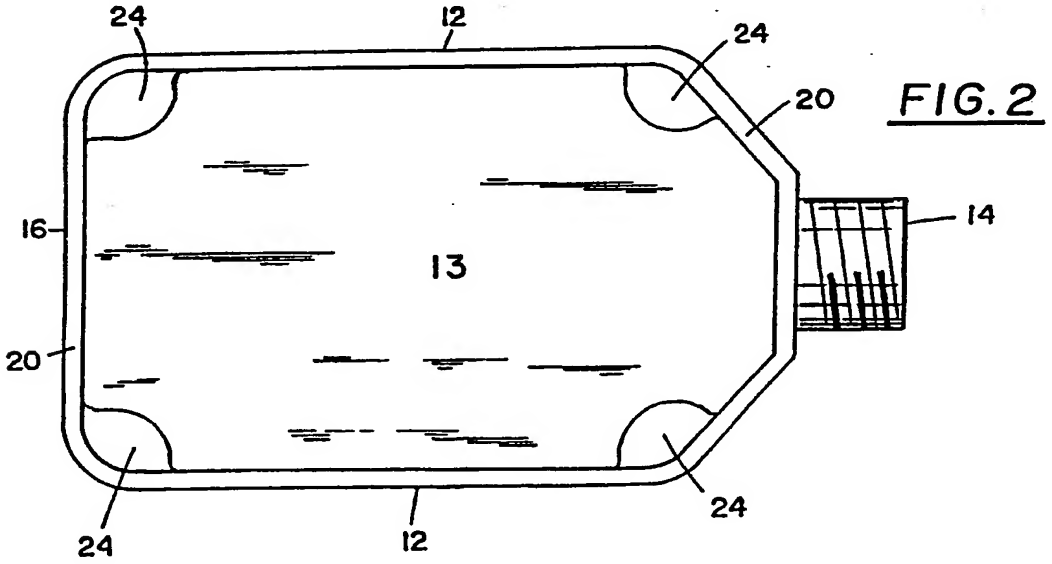
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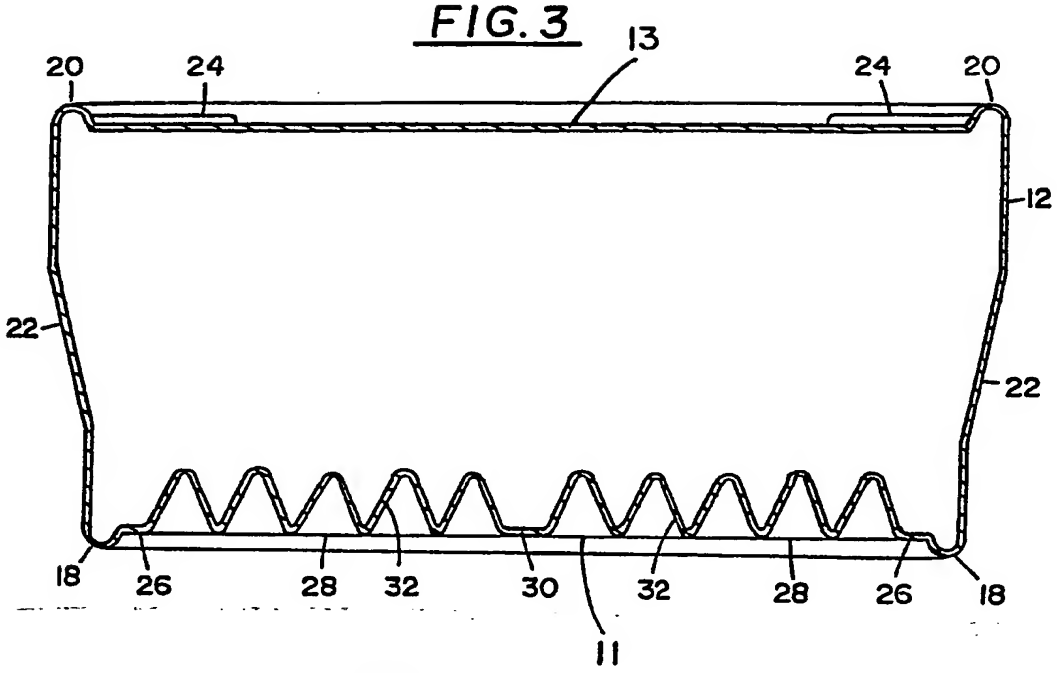
FIG. 2

FIG. 3



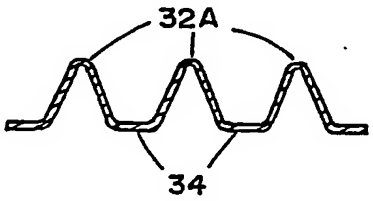


FIG. 4

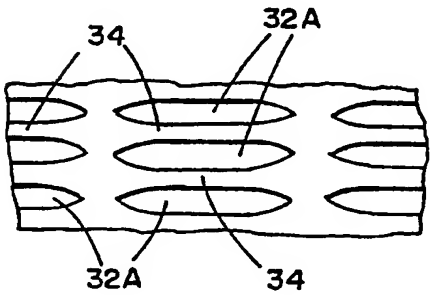
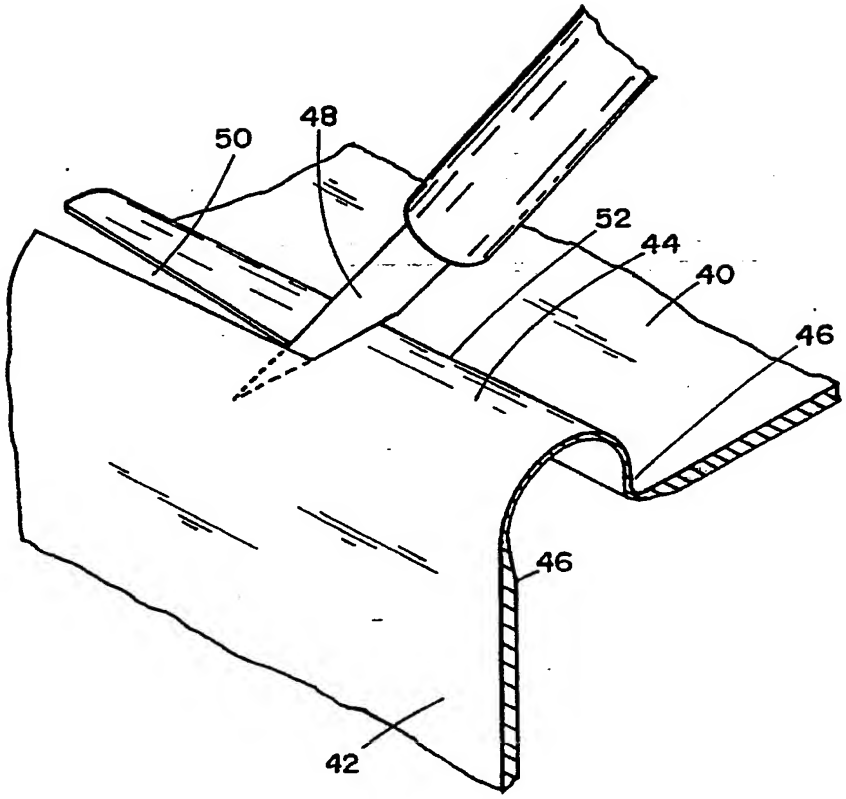


FIG. 5

FIG. 6



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/04375

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12M 3/04, 1/24; C12N 5/00
US CL :435/240.23, 285
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.23, 284-286, 296-299, 310; 422/102; 215/1C, 1R; 220/670-673, DIG.12, DIG.13, DIG.14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 3,870,602 (Froman et al.) 11 March 1975.	1-17
A	US, A, 3,941,661 (Noteboom) 02 March 1976.	1-17
A	US, A, 4,317,886 (Johnson et al.) 02 March 1982.	1-17
A	US, A, 4,770,854 (Lyman) 13 September 1988.	1-17
A	US, A, 4,824,787 (Serkes et al.) 25 April 1989.	1-17
A	US, A, 4,829,004 (Varani et al.) 09 May 1989.	1-17
A	US, A, 4,912,048 (Smith et al.) 27 March 1990.	1-17
A	US, A, 4,912,058 (Mussi et al.) 27 March 1990.	1-17
A	US, A, 4,962,033 (Serkes et al.) 09 October 1990.	1-17
A	US, A, 5,010,013 (Serkes et al.) 23 April 1991.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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